REMARKS

Claims 1-15 are under examination; claims 16 and 17 have been withdrawn. Claim 1 has been amended above to insert a missing word. Claims 5, 6, 7, 9, 10, 11, 13, 14 and 15 have been amended to correct improper multiple dependencies.

Claims 1-15 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite on several different grounds. More specifically claim 1, step (c) was said to be indefinite as a word was missing. This typographical error has been corrected above, thereby obviating this ground of rejection. In addition, claims 5-15 were found to be indefinite on the basis that they were improper multiple dependent claims. Appropriate amendments have been made above to correct the claims dependencies. The examiner also asserted that the subject matter of claim 8 was indefinite in that the claim refers to the detection of target proteins fused to tags by enzymatic reaction, whereas claim 1, from which it depends, specifically states that the target protein is not detected on the basis of its own enzymatic activity. Applicants note that claim 1 specifically excludes the possibility that the target protein itself is detected on the basis of its own enzymatic activity, whereas claim 8 encompasses the possibility that the target protein can be detected by virtue of the enzymatic activity possessed by a tag fused to the target

protein. Thus, the target protein itself does not act as the substrate in any enzymatic detection method; only the tag fused to the target can act in such a way. This is explained in the specification on page 5, last paragraph.

Finally, the examiner found claim 11 to be indefinite on the basis of its recitation of "colonies," as claim 1 recites only one colony or cells originating from a single colony, and because the concepts of colony transfer and the timing of colony transfer also are uncertain as claimed. With regard to the first of these points, Applicants have amended claim 11 above to replace "colonies" with "colony" and thus align the language of the claim with that of claim 1. With regard to the examiner's concerns regarding colony transfer, Applicants are uncertain as to the real basis of the examiner's concern. Claim 11 simply recites an additional step in the method set forth in claim 1, whereby the colony is lifted from growth media on the filter used in step (b). That is, the colony either can be lifted by blotting the colony onto the filter (the colony in this instance grows directly on growth media and the filter paper is applied from above) or the colony can be grown directly on the filter paper which is on top of the growth media and can be lifted off on the filter paper.

Claims 1, 2 and 4-15 have been rejected under 35 U.S.C. § 102 (b) as anticipated by U.S. Patent 5,506,121, issued to Skerra et al. The examiner asserted that the '121 patent discloses a "filter sandwich test" in a method for identifying a cell colony which expresses a soluble target protein, including the steps of growing cell colonies on a first filter or nitrocellulose membrane, filtering the lysate through the filter and detecting the protein which has passed through with an antibody. This rejection is traversed.

Applicants respectfully submit that the '121 patent does not anticipate the claimed invention. Specifically, the '121 patent does not disclose a lysis step and no lysis of the cell colonies is carried out. In addition, the '121 patent is not concerned with a method of identifying a cell colony which expresses a soluble variant of a target protein of interest. Instead, it focuses on the development of a new tag which can be expressed with a protein of interest and which has an affinity for streptavidin. The tag can be used to enable detection and identification of proteins. Thus, the reference does not disclose that a colony expressing a soluble variant of a target protein can be detected.

In making the rejection, the examiner pointed to Example 1 in columns 6 and 7 of the '121 patent, which discloses a filter

sandwich test, as disclosing the method steps of claim 1 of the present application. Applicants respectfully submit that the examiner has misinterpreted this example. Example 1 discusses a filter sandwich test in which *E. coli* are transformed with an expression cassette which expresses a protein (D1.3Fv antibody fragment) labeled with the newly developed tag. The cells then are plated out on a nitrocellulose membrane which lies on an agar plate. The plate is incubated for 8 hours until small colonies are formed on the nitrocellulose membrane. A second filter (or capture membrane) is prepared in parallel by coating with a "solution of 5 mg/ml lysozyme from chicken egg white (Sigma) and the antigen of the D1.3 antibody." The lysozyme used for coating thus is the antigen which will bind to the Fv antibody fragments to be expressed by the *E. coli* colonies. The lysozyme, therefore, is being used as the corresponding antigen to bind the expressed antibody and is not being used for the purpose of lysis (and does not lyse the *E. coli* cells). The capture membrane further is blocked and washed and impregnated with LB media and IPTG. The capture membrane then is placed on an agar plate and the first membrane with the *E. coli* colonies is placed on top of the capture membrane. This is incubated overnight to allow expression of the protein (Fv fragments with the tag). The Fv fragments expressed by *E. coli* are secreted from the cells,

diffuse through the first membrane and bind to the lysozyme antigen on the second (capture) membrane. The proteins are detected by virtue of the peptide tag attached thereto which can bind streptavidin.

Thus, the method disclosed in Example 1 of the reference does not disclose a cell colony lysis step using a lysozyme, as the lysozyme is only present on the second filter to provide an antigen to bind the expressed Fv antibody fragments. Although this may not be completely clear from the disclosure in column 7, lines 1-3, of the '121 patent, wherein it is stated that a second nitrocellulose filter membrane "was coated for 6 hours with a solution of 5 mg/ml lysozyme from chicken egg white (Sigma) and the antigen of the D1.3 antibody," it is quite clear from the reference cited at the beginning of Example 1 of the patent. The procedure of Example 1 was said to follow the strategy previously described by Skerra et al., *Anal. Biochem.* 196:151-155 (1991), a copy of which is enclosed herewith as Exhibit A. This publication discloses the filter sandwich test in greater detail and indicates at page 153, left hand column, that "[w]e have attempted to optimize the expression of functional Fab fragments using the fragments D1.3 and NQ11 with specificities for a protein antigen (lysozyme)...." Thus, the lysozyme is coated on the second membrane in Skerra et al. in order to bind the D1.3

fragments produced by the *E. coli* cells and is not present to lyse the cells. Although the method in the Skerra et al. paper is slightly different from the method of the cited patent (the secreted proteins produced by the colonies are bound on the second filter by anti-globulin and then are detected by adding lysozyme conjugated to biotin), it can be seen that the lysozyme is not used in either document to lyse the cells and is only used as an antigen for binding to the Fv fragments expressed from *E. coli*.

In addition, the lack of a lysis step in the '121 patent is further supported by the statement in column 7, lines 15-18, which provides that the first membrane with the cell colonies was finally placed on a fresh LB agar plate in order to store the colonies for further proliferation. This indicates that the colonies have not been lysed. Furthermore, the patentees do not explicitly discuss lysis anywhere in the document and only refer to the second filter as an "antibody capture membrane." It is not indicated that the second filter has an additional purpose of lysing the cells. From this, it is apparent that the only function of the lysozyme on the second filter is as an antibody capture membrane as disclosed in the Skerra et al. paper. There thus is no lysis step in the '121 patent and the method of the present claims is novel.

In describing the '121 patent, the examiner noted on page 4 of the Action that it discloses the application of a force, since generic gravitational force would be present to pull the proteins through the filter. Applicants respectfully submit that the examiner's assertions are incorrect. As an initial point, Applicants submit that the gravitational force on proteins is negligible, as the proteins are so small and their weight is very low. Gravitational force thus would not be able to pull the proteins through the filter. In addition, Applicants wish to point out that claim 13 specifies the application of a force to the filter which indicates that an additional force is applied which is not already present. Such forces are described on page 11, second paragraph, of the specification. A skilled person would understand that the application of a force would not include gravity.

The examiner also seems to be of the impression that the peptide tag of the '121 patent is a His tag. Applicant respectfully submit that one of skill in the art would not consider the peptide tag of the '121 patent to be a His tag as defined in claim 7 of the present application. The peptide tag of the '121 patent only explicitly comprises a single His residue. This would not constitute a His tag, which is a well-known term used in the art and which requires the presence of at

least 2 His residues to bind IMAC. The tag of the '121 patent thus is not a "His tag."

Claims 1-15 have been rejected under 35 U.S.C. §102(b) as anticipated by Knaust et al., *Anal. Biochem.* 297:79-85 (2001). The examiner asserted that the lysis step in this reference falls within the scope of the present claims and he indicates that the reference discloses a step of "subjecting a cell originating from a single cell colony to conditions which are capable of causing lysis." This rejection is traversed.

The method of the present invention requires the lysis of a cell colony. A cell colony is defined in the bridging paragraph between pages 6 and 7 of the specification and clearly provides that a colony is a circumscribed group of cells growing on a solid or semi-solid medium. Lines 17-18 on page 7 reinforce that "'colonies' do not encompass cells grown in liquid culture." This is in contrast to the teachings of Knaust. In the Knaust reference, cells are lysed when in liquid medium and, therefore, direct cell colony lysis is not disclosed or contemplated. The Knaust reference, therefore, does not anticipate the present claims.

Claims 1-15 have been rejected under 35 U.S.C. §103(a) as unpatentable over the '121 patent and the Knaust reference. The examiner asserted that the two references teach or suggest all

claimed limitations and that it would have been obvious to apply either lysing buffer or freeze-thawing cells in order to lyse cells and to liberate soluble proteins with a reasonable expectation of success in filtrating and detecting soluble proteins in the method of identifying cell colonies expressing soluble target proteins as taught or suggested by the references. This rejection is traversed.

Neither of the two cited references discloses or suggests that lysis of cells and filtration of lysates can be carried out directly on colonies of cells. This direct lysis step, which is not taught or suggested in either document, has enabled the development of the present method, which can operate on large numbers of variants, is inexpensive and has a high reliability in predicting soluble variants of proteins.

The '121 patent is not concerned with the identification of soluble protein variants, and the patent does not suggest that soluble protein variants can be screened using a filtration method of the present invention which uses a direct lysis step on colonies. One of skill in the art would not be motivated to develop the method of the invention from the teachings of the '121 patent. The patent does not indicate that soluble proteins can be screened (i.e., distinguished from insoluble proteins) by using filtration. Nor does the '121 patent teach a lysis step,

much less a step of direct colony lysis. The proteins produced in accordance with the teachings of the '121 patent are secreted. One of skill in the art would not appreciate from the teachings of the '121 patent that a soluble protein screen could be developed which would employ a step of direct colony lysis.

The teachings of the Knaust reference do not compensate for the shortcomings of the '121 patent. The method taught in this reference includes multiple pipetting steps, which is very time-consuming, as cell lysis is carried out in liquid, which is not contemplated by the present invention. There is no teachings or suggestion in the Knaust reference as to how the method could be improved, and there certainly is no indication that direct colony lysis could be used or that such a technique would revolutionize the method. The present invention has enabled the development of an extremely efficient screening tool which was not even alluded to in the prior art. The cited references do not render obvious the present invention.

In view of the foregoing amendments and discussion, Applicants respectfully submit that the claims of this application are in condition for allowance.

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